Spatial toxicity of selected insecticidal plant oils against Anopheles gambiae Giles (Diptera: Culicidae)

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Abstract

Background

Plant oils possess biological activities which offer tremendous potential for disease vector management. This study investigated the bioactivity of selected insecticidal plant oils against *Anopheles gambiae* s.s. Kisumu Susceptible Strain (KSS) mosquitoes. The plant oils from leaves of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides* and fruit peels of *Citrus sinensis* were extracted and the major chemical components analysed using coupled gas chromatography-mass spectrometry (GC-MS). The spatial toxicity effects of the plant oils were tested at different concentration (0.1 mg/ml, 0.3 mg/ml and 0.5 mg/ml) against *An. gambiae* s.s. KSS in a Peet-Grady Chamber. Deltamethrin was used as the positive control.

Results

D-Limonene was the major chemical component found in *Ci. sinensis* and *Ni. tabacum*. Prococene I was the major chemical component in *Oc. gratissimum* while P-Xylene had the highest percent composition in *Ni. tabacum*. The control (Deltamethrin) and 0.3 mg/ml concentration of oil from *Oc. gratissimum* elicited a 100% knockdown on *An. gambiae* s.s. KSS. At 0.5 mg/ml, oils from *Ci. sinensis, Oc. gratissimum* and *Ni. tabacum* elicited significantly ($P > 0.05$) the same knockdown effect (100%) as the control on *An. gambiae* s.s. KSS. After 24hrs post-exposure, all the plant oils evaluated elicited 100% mortality on *An. gambiae* s.s. KSS at both 0.3 and 0.5 mg/ml. The lowest knockdown times KdT_{50} (34.80 minutes) and KdT_{95} (92.50 mins) were noted at the lowest concentration (0.1 mg/ml) of oils from *Ni. tabacum* which was better compared to the control (36.1 and114.9 mins respectively) against *An. gambiae* s.s. KSS.

Conclusions

Oc. gratissimum and *Ni. tabacum* displayed excellent spatial toxicity capabilities against *An. gambiae* s.s. KSS and can be incorporated into the management of malaria vectors in Nigeria.

Background

The health risks associated with vector-borne diseases have long encouraged research into methods for protection in endemic areas, in both the grassroots and scientific communities (Karunamoorthi, 2013). Diligent investigations into personal protection methods using plant extracts and oils by the scientific community have provided new bio-rational, effective and affordable products, increasing knowledge and confidence in neglected traditional methods of protection from insect-borne diseases (Dube et al., 2011). Plant oils possess a wide spectrum of biological characteristics including anti-microbial, fungicidal, herbicidal, acaricidal, nematocidal, insecticidal and insect repellent activities (Noutcha et al., 2016) and could be used for vector control.

Mosquitoes have become the most important single group of insects well-known for their public health importance since they act as the vector for many parasites and pathogens in the tropics (Egunyomi et al., 2010). Malaria a foremost mosquito-borne disease is the second leading cause of death from infectious diseases in Africa after Human Immunodeficiency Virus (HIV) and it is the highest cause of death among children under age five in Africa and Nigeria (NDHS, 2018; WHO, 2019). The North-Central geo-political zone has the second-highest malaria prevalence in Nigeria (NDHS, 2018). The major malaria vector in Nigeria involved in the transmission is the *Anopheles gambiae* complex (Okorie et al., 2011; Oduola et al., 2016).

The world's prime choice for mosquito control in Africa still remains the selective application of residual synthetic insecticides through Indoor Residual Spray or Long-Lasting Insecticide Nets which have been highly effective in reducing mosquito-borne disease burden yielding large scale results at an affordable cost (WHO, 2012). However, the increasing occurrence of insecticide resistance in *Anopheles* mosquito populations to commonly used insecticides has been reported (Oduola et al., 2019). Hence, the need to effectively manage these insect vectors by seeking additional methods that can effectively reduce the burden they impose on the populace. Insecticidal plants have been used in many parts of the world for killing or repelling mosquitoes either as whole plants, extracts or oils because of their toxicity potential (Karunamoorthi et al., 2009, Youmsi et al., 2017).

Mosquitoes elicit varying behaviour in response to airborne components which involves avoidance of a chemical stimulus, loss of host detection, knockdown and mortality (Ogoma et al., 2014). Spatial toxicity does not require physical contact of the mosquito with treated surfaces since the toxicant can act in the vapour state and at a distance (Ogoma et al., 2014). Examples of spatial toxicants include; Mosquito coils, candles and emanators impregnated with volatile pyrethroids and plant oils. Plant oils can be used as spatial toxicants by applying them in their fresh state or as fumigants against *Anopheles* mosquitoes both indoors and outdoors (Ileke et al., 2015; Ukpong et al., 2016). Insecticidal plants possess and exhibit excellent spatial toxicity capabilities. This study was designed to evaluate the spatial toxicity of plant oils from leaves of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides* and fruit peel of *Citrus sinensis* against the reference *Anopheles gambiae* s.s. KSS.

Methods

Mosquito Rearing

Adult Female *Anopheles gambiae* s.s. Kisumu Susceptible Strain (KSS) maintained at the Nigeria Institute of Medical Research, Lagos insectary was used for the study. The susceptible strains are being maintained in the insectary under standard insectary conditions (WHO, 2005) i.e., 27 ± 2^0c and 85 ± 5% Relative humidity. The larvae stages were reared in 24 x 18 x 4 cm plastic larvae trays containing 500 ml distilled water and fed non-fatty biscuit and yeast at 3:1 while the adults were reared in a 50 x 50 x 50 cm mosquito cage and fed 10% sugar solution.

Extraction of Plant Oils

The leaves of *Hyptis suaveolens, Ocimum gratissimum, Nicotiana tabacum, Ageratum conyzoides* and fruit peel of *Citrus sinensis* were air-dried under shade for one week in the Entomology Laboratory, Department of Zoology, University of Ilorin before hydro-distillation. The plant oils were extracted by steam distillation using a Clevenger apparatus. The extracted plant oils were dried over anhydrous sodium sulphate and stored in amber coloured vials at 4^0C until required for assays. Before evaluation, the plant oils were prepared in three concentrations: 10% (vol:vol) – 0.1 mg/ml, 30% (vol:vol) – 0.3 mg/ml, and 50% (vol:vol) – 0.5 mg/ml using technical grade acetone. These concentrations were determined based on previous unpublished data.

Evaluation of Major Chemical Components

Coupled gas chromatography-mass spectrometry analysis (GC-MS) of the extracted plant oils was performed on a Hewlett Packard 5890 II gas chromatograph, interfaced to a single quadrupole mass selective detector (Model 5972). The column was an HP-5 MS capillary column (30 × 0.25 mm, film thickness 0.25 mm). Helium was the carrier gas, set at a flow rate of 0.6 ml/min. Injector and MS transfer line temperatures were set at 220 and 250°C, respectively. The oven programme temperature was held at 35°C for 5 mins and then 4°C/min to 150°C for 2 mins and then finally 20°C/min to 250°C for 5 mins. Diluted samples (10:100 in CH_2C_{l2}, v/v) of 1 µL were injected manually and in a split mode (1:100 split ratio). The identification of the components was accomplished by comparison of their relative retention indices as well as comparison of mass spectra with those of standards, those found in the literature and those supplemented by National Institute of Standards and Technology (NIST) provided by Hewlett Packard with the GC/ MS control and data processing software.

Spatial Toxicity Assay

This assay was conducted in a Peet-Grady Chamber measuring 180 x 180 x 180 cm. A total of 100 laboratory-cultured adult female, sucrose-fed susceptible Kisumu *An. gambiae* s.s. age 2 to 5 days were released into the chamber from the release ports (WHOPES, 2009). The following concentrations 0.1 mg/ml, 0.3 mg/ml and 0.5 mg/ml of the plant oils were applied to 110mm diameter Whatman filter paper using a pipette as described by Dua et al. (2008). Thereafter, the Whatman paper with a concentration of the treatment was placed at the centre of the chamber and the fan circulating air for the chamber was turned on. Knockdown mosquitoes were observed every 10 minutes for 60 minutes. After 60 minutes, all mosquitoes were then collected and placed in a clean well-labelled paper cup and the mosquitoes were provided with 10% sugar solution. Mortality was observed after 24 hours post-treatment. All tests were conducted at a temperature of 27 ± 2º C and relative humidity of 80 ± 10%. Deltamethrin (Centre for Disease Control, Stock Solution: 10 mg/ml, Diagnostic Dose: 12.5ug/BT) was used as the positive control. The assay was replicated four times for each treatment (WHOPES, 2009).

Data Analysis

Percentage knockdown was calculated using the formula below:

$$\% \text{ Knock Down} = \frac{\text{Total number of knockdown mosquitoes after 60minutes}}{\text{Total number of exposed mosquitoes}} \times 100$$

Analysis of Variance (ANOVA) and the Tukey test were used to compare the mortality rates and Knockdown Times (KdT) in the treatments and control for the spatial toxicity assay. The KdT_{50} and KdT_{95} were determined with the aid of GraphPad Prism 8, using the probit analysis (Finney, 1971).

Results

Chemical constituent of selected plant oils

The percentage composition of major chemical components from the GC-MS analysis of oil from *Hyptis suaveolens, Citrus sinensis, Ocimum gratissimum, Nicotiana tabacum* and *Ageratum conyzoides* are shown in Table 1. D-Limonene was a major chemical component found in two of the five plant oils evaluated namely *Ci. sinensis* (92.5%) and *Ag. conyzoides* (63.96%) and occurred in trace amounts in *Oc. gratissimum* (2.62%) while 3-Octen-1-ol occurred in trace amounts in *Hy. suaveolens* (1.09%) and *Ci. sinensis* (0.97%). However, Precocene I (95.63%) was the major component found in *Oc. gratissimum* and Caryophyllene (0.92%) occurred in trace amounts while P-Xylene (12.37%) and Farnesol (3.08%) occurred in trace amounts in *Ni. tabacum*.

Table 1
Percentage Composition of Major chemical constituents of selected plant oils.

s/n	Component	*Hyptis suaveolens*	*Citrus sinensis*	*Ocimum gratissimum*	*Nicotiana tabacum*	*Ageratum conyzoides*
1.	D-Limonene		92.5	2.62		63.96
2.	3-Octen-1-ol, (Z)	1.09	0.97			
3.	Precocene I			95.63		
4.	Caryophyllene			0.92		
5.	1-Octyn-3-ol	9.81				
6.	P-Xylene				12.37	
7.	Farnesol			0.05	3.08	
8.	Preg-4-en-3-one			0.05		0.58
9.	Paromomycin	1.20				

Knockdown effect of selected plant oils on Anopheles gambiae **s.s. KSS**

The percentage knockdown after 60 minutes of exposure ranged from 58.3–66.7%, 90-96.7% and 95-98.3% from 0.1 mg/ml, 0.3 mg/ml and 0.5 mg/ml of oils from *Hy. suaveolens* and *Ag. conyzoides* on *An. gambiae* which was lower than what was observed in the control (Deltamethrin) (100%) after 60 minutes of exposure (Fig. 1a & e). After 60 minutes, 0.3 mg/ml of oils from *Oc. gratissimum* achieved a 100% knockdown which was the same as the control (Fig. 1c). However, 0.1 mg/ml and 0.3 mg/ml of oils from *Ci. sinensis* and *Ni. tabacum* had a lower percentage knockdown (68.3–71.7% and 90-96.7%) on *An. gambiae* compared to the control (100%) after 60 minutes of exposure (Fig. 1b & d). Meanwhile, 0.5 mg/ml of oils from *Ci. sinensis, Oc. gratissimum* and *Ni. tabacum* elicited significantly the same effect ($P > 0.05$) as the control (100%) on *An. gambiae.* There was no significant difference ($P > 0.05$) between the percentage knockdown on *An. gambiae* observed in 0.5 mg/ml of oil from *Hy. suaveolens, Ci. sinensis, Oc. gratissimum, Ni. tabacum* and *Ag. conyzoides* and the control.

Percentage Mortality and Knock Down Times of Oils from Selected Plants on Anopheles gambiae **s.s. KSS**

Concentrations of 0.3 mg/ml and 0.5 mg/ml of all the five plant oils evaluated (*Ci. sinensis*, *Hy. suaveolens*, *Ni. tabacum*, *Ag. conyzoides* and *Oc. gratissimum*) achieved 100% mortality on *An. gambiae* s.s. KSS which was the same percentage mortality achieved in the control after 24 hours. There was no significant difference ($P > 0.05$) between the mortality rates of the control and 0.3 mg/ml and 0.5 mg/ml concentrations of the plant oils evaluated. However, it was observed that the 0.1 mg/ml concentration of all the five plant oils evaluated did not achieve 100% mortality. There was a significant difference ($P < 0.05$) between 0.1 mg/ml concentration of all the five plant oils and the control (Table 2).

At 0.1 mg/ml, *Ni. tabacum* (34.80 mins), demonstrated low KdT_{50} compared to the control (36.10 mins), *Hy. suaveolens* (44.48 mins), *Oc. gratissimum* (58.72 mins), *Ag. conyzoides* (66.27 mins) and *Ci. sinensis* (73.08 mins). Meanwhile at 0.3 mg/ml, *Ag. conyzoides* (33.47 mins), demonstrated low KdT_{50} compared to *Oc. gratissimum* (36.68 mins) and *Ni. tabacum* (38.70 mins). At 0.5 mg/ml, *Oc. gratissimum* (21.32 mins), demonstrated low KdT_{50} compared to *Hy. suaveolens* (25.18 mins) (Table 2).

At 0.1 mg/ml, *Ni. tabacum* (92.50 mins), demonstrated low KdT_{95} compared to the control (114.90 mins), *Hy. suaveolens* (163.20 mins), *Oc. gratissimum* (172.20 mins), *Ag. conyzoides* (372.60 mins) and *Ci. sinensis* (403.20 mins). At 0.3 mg/ml, *Oc. gratissimum* (27.17 mins), demonstrated low KdT_{50} compared to *Ni. tabacum* (95.58 mins) and *Ag. conyzoides* (127.60 mins). At 0.5 mg/ml, *Oc. gratissimum* (37.05 mins), demonstrated low KdT_{50} compared to *Hy. suaveolens* (73.49 mins) (Table 2). Both knockdown times (KdT_{50} and KdT_{95}) indicated that *Ni. tabacum* had the lowest time to knock down 50% and 95% of the *An. gambiae* population. Other oils such as *Hy. suaveolens*, *Oc. gratissimum*, *Ag. conyzoides* and *Ci. sinensis* followed in this order Table (2).

In terms of percentage mortality induced on exposed *An. gambiae* mosquitoes, *Ci. sinensis* induced the highest mortality (85%) followed by *Oc. gratissimum* (83%), *Ni. tabacum* (82%), *Ag. conyzoides* (77%) and *Hy. suaveolens* (68%) at 0.1 mg/ml. Only exposure to the positive control resulted in 100% mortality

and none of the plant oils at 0.1 mg/ml produced more than 85% mortality after 24 hours post-exposure. Meanwhile, at 0.3 and 0.5 mg/ml post-exposure mortality of all the plant oils evaluated on *An. gambiae* after 24 hours, was 100%.

Table 2
Knockdown values and Percentage Mortality of selected plant oils exposed to *An. gambiae*

Plant oils	Conc. (mg/ml)	Number of mosquitoes exposed	KdT_{50} (95% CI)	KdT_{95} (95% CI)	% Mortality after 24 hrs
Hyptis suaveolens	0.1	100	44.48 (18.73–105.6)	163.2 (17.78–1498)	68_a
	0.3	100	NA	NA	100_b
	0.5	100	25.18 (17.89–35.43)	73.49 (17.27–312.8)	100_b
Citrus sinensis	0.1	100	73.08 (0.218–24497)	403.2 (0.008-NA)	85_a
	0.3	100	NA	NA	100_b
	0.5	100	NA	NA	100_b
Ocimum gratissimum	0.1	100	58.72 (18.33–188.1)	172.2 (18.42–1610)	83_a
	0.3	100	36.68 (13.25–101.5)	271.7 (5.693–12970)	100_b
	0.5	100	21.32 (19.99–22.74)	37.05 (29.94–45.85)	100_b
Nicotiana tabacum	0.1	100	34.8 (21.83–55.48)	92.51 (19.84–431.4)	82_a
	0.3	100	38.7 (28.29–52.95)	95.58 (37.71–242.3)	100_b
	0.5	100	NA	NA	100_b
Ageratum conyzoides	0.1	100	66.27 (5.297–829.2)	372.6 (2.568–54055)	77_a
	0.3	100	33.47 (24.30-46.12)	127.6 (39.08–416.5)	100_b
	0.5	100	NA	NA	100_b

Plant oils	Conc. (mg/ml)	Number of mosquitoes exposed	KdT_{50} (95% CI)	KdT_{95} (95% CI)	% Mortality after 24 hrs
Positive Control (Deltamethrin)	10	100	36.1 (13.55–96.19)	114.9 (4.899–2697)	100_b
Negative Control (Acetone)	NA	100	NA	NA	0_c

Conc.- Concentration, KdT – knockdown time, NA – Not Applicable, % Mortality-Percentage mortality after 24hrs, Values along column with different subscripts differ significantly ($P > 0.05$)

Discussion

In this study, the chemical constituents, knockdown and mortality potentials of five insecticidal plants were established. All the concentrations of oils of plants (*Hyptis suaveolens*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Citrus sinensis* and *Nicotiana tabacum*) shortlisted from preliminary profiling (Adelaja et al., 2021) were evaluated for their major chemical constituents and spatial toxicity potential.

The study identified D-Limonene as one of the major chemical components in oils from *Ci. sinensis*, *Ag. conyzoides* and *Oc. gratissimum*. It has been reported that D-Limonene is a key chemical component in oils from *Ci. sinensis, Ag. conyzoides* and *Oc. gratissimum* (Ahmad et al., 2006; Espina et al., 2011). The isolated form of D-Limonene has been reported to have insecticidal potential against stored grain insects (e.g., *Sitophilus oryzae, Tribolium castaneum, Oryzaephilus surinamensis*) (Lee et al., 2003), termites (Almeida et al., 2015), fleas, mites and wasp (Okwute, 2012). Also, Precocene I and Caryophyllene were identified as major chemical components in oil from *Oc. gratissimum*. Similarly, Caryophyllene was identified as a major chemical component in oils of *Oc. gratissimum* (Joshi, 2017; Saliu et al., 2011; Dambolena et al., 2010). In this study, P-Xylene was identified as a major chemical component in oil from *Ni. tabacum*. This is similar to a report where P-Xylene was identified as the key chemical component in oils from *Ni. tabacum* (Kidah, 2018).

Interestingly, all the five plant oils evaluated elicited good knockdown activity and mortality on *An. gambiae* at the highest concentration evaluated, and they elicited a similar knockdown effect and mortality as the control (Deltamethrin) at the highest concentrations. Meanwhile, oils from *Ci. sinensis*, *Ni. tabacum* and *Oc. gratissimum* elicited the same knockdown effects as the control on *An. gambiae* s.s. KSS after 60 minutes of exposure. Similarly, it was reported that, *Ci. sinensis* one of the plants evaluated was burnt by locals in some rural communities in Africa and it displayed a capability of spatially killing mosquitoes indoors (Manimaran et al., 2012) while an investigation on the fumigant or spatial toxicity effect of *Ni. tabacum* on *An. gambiae* and recorded a hundred percent mortality (Ileke et al., 2015).

Oc. gratissimum elicited the best knockdown effect at the lowest concentration which was comparable to activities reported in the control. Similarly, a report showed that *Oc. gratissimum* elicited a good

knockdown effect on *An. gambiae* after 60 minutes of exposure. *Ni. tabacum* displayed the best toxicity in terms of knockdown time against *An. gambiae* s.s. KSS (Ileke and Adeshina 2019). Similarly, in another study, rooms inhabited by tobacco smokers and non-smokers were evaluated and it was reported that there were significantly lower mosquitoes in the rooms inhabited by smokers compared to non-smokers (Obembe et al., 2018). The toxicity activity recorded from *Ci. sinensis* and *Oc. gratissimum* must be as a result of the chemical constituent present which is D-Limonene while for *Ni. tabacum* it might be P-Xylene. This study establishes that oils from *Ci. sinensis*, *Ni. tabacum* and *Oc. gratissimum* could be employed as spatial toxicants indoors for personal protection purposes against malaria vectors and have the potential to supplement the current interventions in play that targets anthropophilic, endophilic and endophagic mosquitoes in areas where there are reports of resistance development to public health insecticides.

Interestingly, a hundred percent mortality was observed in 0.3 mg/ml concentrations of all the plant oils evaluated on *An. gambiae* s.s. KSS after 24 hours displays their potential to be incorporated into an indoor residual spray or house paints.

Lower KdT_{50} and KdT_{95} values were observed in *An. gambiae* s.s. KSS exposed to oils from *Oc. gratissimum* and *Hy. suaveolens* show a better knockdown effect compared to Deltamethrin. *Oc. gratissimum* and *Hy. suaveolens* could be used for the management of outdoor biting *Anopheles* mosquitoes in places where they have been reported to have developed resistance to commonly used insecticides for vector control interventions and there are concerns with the use of Deltamethrin

Conclusions

This study established the spatial toxicity potential of five plant oils against *An. gambiae* s.s. KSS elucidating their potential for malaria vector management in Nigeria. Plant oils from *Oc. gratissimum* had the best knockdown effect while *Ni. tabacum* elicited the best toxicity against *An. gambiae* s.s. KSS reiterates their efficacy against malaria mosquitoes. There is a need to isolate identified chemical components and evaluate their bioactivity on *Anopheles* mosquitoes.

Abbreviations

KSS
Kisumu Susceptible Strain
GC-MS
coupled gas chromatography mass spectrometry
KdT
Knockdown Times

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the Faculty of Life Science ethical committee before the onset of the research with the University Ethical Review Committee Approval Number of UERC/LSC/119.

Consent for publication

Not Applicable

Availability of data and materials

The datasets generated during and/or analysed during the current study are not publicly available due (General agreement among the authors) but are available from corresponding author on reasonable request.

Competing interests

The Authors declare that they have no competing interest.

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Authors' contributions

Authors OJA and AOO conceptualise this research concept and designed the research protocol and methodology. Authors OJA and AIO carried out the field work. Author AO and AOA provided technical input. Author OJA did the statistical analysis and developed the manuscript. All the authors read, corrected and approved the final manuscript.

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References

1. Adelaja, O. J., Oduola, A. O., Abiodun, O. O., Adeneye, A. K., & Obembe, A. (2021). Plants with insecticidal potential used by ethnic groups in North-Central Nigeria for the management of hematophagous insects. *As Jour Ethnobiol*, *4*, 65–75
2. Ahmad, M. M., Rehman, S., Iqbal, Z., Anjum, F. M., & Sultan, J. I. (2006). Genetic variability to essential oil composition in four Citrus fruit species. *Pakis Jour Bota*, *38*, 319–324

3. Almeida, M. L. S., Oliveira, A. S., Rodrigues, A. A., Carvalho, G. S., Silva, L. B., Lago, J. H. G., & Casarin, F. E. (2015). Antitermitic activity of plant essential oils and their major constituents against termite *Heterotermes sulcatus* (Isoptera: Rhinotermitidae). *Jour Medi Pla Res*, *9*, 97–103
4. Dambolena, J. S., Zunino, M. P., Lopez, A. G., Rubinstein, H. R., Zygadlo, J. A., et al. (2010). Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by Fusarium verticillioides. *Innovat Food Sci Emerg Technol*, *11*, 410–414
5. Dua, V. K., Alam, M. F., Pandey, A. C., Rai, S., Chopra, A. K., Kaul, V. K., et al. (2008). Insecticidal activity of *Valeriana jatamansi* (Verbenaceae) against mosquitoes. *Journal Of The American Mosquito Control Association*, *24*, 315–318
6. Dube, F. F., Tadesse, K., Birgersson, G., Seyoum, E., Tekie, H., Ignell, R., & Hill, S. R. (2011). Fresh, dried or smoked? Repellent properties of volatiles emitted from ethnomedicinal plant leaves against malaria and yellow fever vectors in Ethiopia. *Mala Jour*, *10*(1), 375. https://doi.org/10.1186/1475-2875-10-375
7. Egunyomi, I. T., Gbadamosi, I. T., & Osiname, K. O. (2010). Comparative effectiveness of ethnobotanical mosquito repellents used in Ibadan, Nigeria. *Journ App Biosci*, *36*, 2383–2388
8. Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., & Pagán, R. (2011). Chemical composition of commercial Citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Cont*, *22*, 896–902. http://dx.doi.org/10.1016/j.foodcont.2010.11.021
9. Finney, D. J. (1971). *"Probit Analysis*. Cambridge: " Cambridge University Press
10. Ileke, K. D., & Adesina, J. M. (2019). Toxicity of *Ocimum basilicum* and *Ocimum gratissimum* Extracts against Main Malaria Vector, *Anopheles gambiae* (Diptera: Culicidae) in Nigeria. *Jour Arthrop Dis*, *13*(4), 362–368
11. Ileke, K. D., Ogungbite, O. C., et al. (2015). *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *Jour Coas Lif Med*, *3*(7), 557–563
12. Joshi, R. K. (2017). GC – MS Analysis of the Essential Oil of *Ocimum gratissimum* L. Growing Desolately in South India. *Acta Chromatographica*, *29*, 111–119. https://doi.org/10.1556/1326.2017.29.1.10
13. Karr, L. L., & Coats, J. R. (1988). Insecticidal properties of d-limonene. *Jour Pestic Sc*, *13*, 287–290
14. Karunamoorthi, K. (2013). Yellow Fever Encephalitis: An Emerging and Resurging Global Public Health Threat in a Changing Environment. In Encephalitis. Edited by Sergey T. InTech Publisher Available from: http://www. intechopen.com/books/encephalitis/yellow-fever-encephalitis-an-emergingand-resurging-global-public-health-threat-in-a-changing-enviro. ISBN 978-953-51-0925-9
15. Karunamoorthi, K., Ilango, K., & Endale, A. (2009). Ethnobotanical survey of knowledge and usage custom of traditional insect/mosquito repellent plants among the Ethiopian Oromo ethnic group. *Jour Ethnopharm*, *125*, 224–229

16. Kidah, M. I. (2018). Chemical Constituents of the Essential Oil Extracted from *Nicotiana tabacum* Leaves. *Biotech Jour Intern*, *21*(1), 1–4. https://doi.org/10.9734/BJI/2018/29911
17. Lee, S., Peterson, C. J., & Coats, J. R. (2003). Fumigation toxicity of monoterpenoids to several stored product insects. *Journ Stored Prod Res*, *39*, 77–85
18. Manimaran, A., Cruz, M., Muthu, C., Vincent, S., & Ignacimuthu, S. (2012). Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). *Adva Biosc Biotech*, *3*, 855–862. doi: 10.4236/abb.2012.37106
19. Nigeria Demographic and Health Survey (NDHS) (2018). http://www.population.gov.ng/index.php/2018-nigeria-demographic-and-health-survey-dissemination-by-state-in-progress Accessed: 24th June 2019
20. Noutcha, M., Edwin-Wosu, N., Ogali, R., & Okiwelu, S. (2016). The Role of Plant Essential Oils in Mosquito (Diptera: Culicidae) Control. *Ann Res Rev Biol*, *10*(6), 1–9. https://doi.org/10.9734/ARRB/2016/28432
21. Obembe, A., Popoola, K. O. K., Oduola, A. O., & Awolola, S. T. (2018). Differential behaviour of endophilic Anopheles mosquitoes in rooms occupied by tobacco smokers and non-smokers in two Nigerian villages. *Jour Appl Sc Envir Manag*, *22*(6), 981–985
22. Oduola, A. O., Abba, E., Adelaja, O. J., Ande, A. T., Yoriyo, K. P., & Awolola, T. S. (2019). *Widespread Report of Multiple Resistance in Anopheles gambiae Mosquitoes in Eight Communities in Southern Gombe, North East Nigeria*. Jour Arthr B Dis
23. Oduola, A. O., Adelaja, O. J., Ayiegbusi, Z. O., Tola, M., Obembe, A., Ande, A. T., & Awolola, S. (2016). Dynamics of Anopheline vector species composition and reported malaria cases during the rain and dry season in two selected communities in Kwara state. *Nig Jour Parasit*, *37*(2), 158–164
24. Ogoma, S. B., Ngonyani, H., Simfukwe, E. T., Mseka, A., Moore, J., et al. (2014). The Mode of Action of Spatial Repellents and Their Impact on Vectorial Capacity of *Anopheles gambiae* sensu stricto. *Plos One*, *9*(12), e110433. doi:10.1371/journal.pone.0110433
25. Okorie, P. N., McKenzie, F. E., Ademowo, O. G., & Bockarie, M. (2011). Nigeria Anopheles vector database: an overview of 100 years' research. *PLoS One*, *6*(12), e28347
26. Okwute, S. K. (2012). Plants as Potential Sources of Pesticidal Agents: A Review. *Pesticides – Advan Chem Bot Pest*, 207–232. https://doi.org/10.5772/46225
27. Saliu, B. K., Usman, L. A., Sani, A., Muhammad, N. O., & Akolade, J. O. (2011).Int. J. Curr. Res., 33,022–028
28. Ukpong, I. G., Ettah, H. E., & Eshuong, E. E. (2016). studies on mosquito repellent activity of *cymbopogon citratus* (lemon grass) using human volunteers. *Inter Jour Res - Grantha*, *4*(12), 41–47. https://doi.org/10.5281/zenodo.221591
29. WHOPES. (2009). *Guidelines for efficacy testing of mosquito repellents for human skin*. Geneva: World Health Organisation
30. World Health Organization (2005). Guidelines for laboratory and field testing of mosquito

31. World Health Organization. (2012). *Handbook for Integrated Vector Management (IVM)*. Geneva: WHO Press. /2012.3
32. World Health Organization (2019). World malaria report. Global malaria programme World Health Organization, Geneva 1–284
33. Youmsi, R. D. F., Fokou, P. V. T., Menkem, E. Z., Bakarnga-Via, I., Keumoe, R., Nana, V., & Boyom, F. F. (2017). Ethnobotanical survey of medicinal plants used as insects' repellents in six malaria endemic localities of Cameroon. *Jour Ethnobio Ethnomed*, *13*(1), 33

Figures

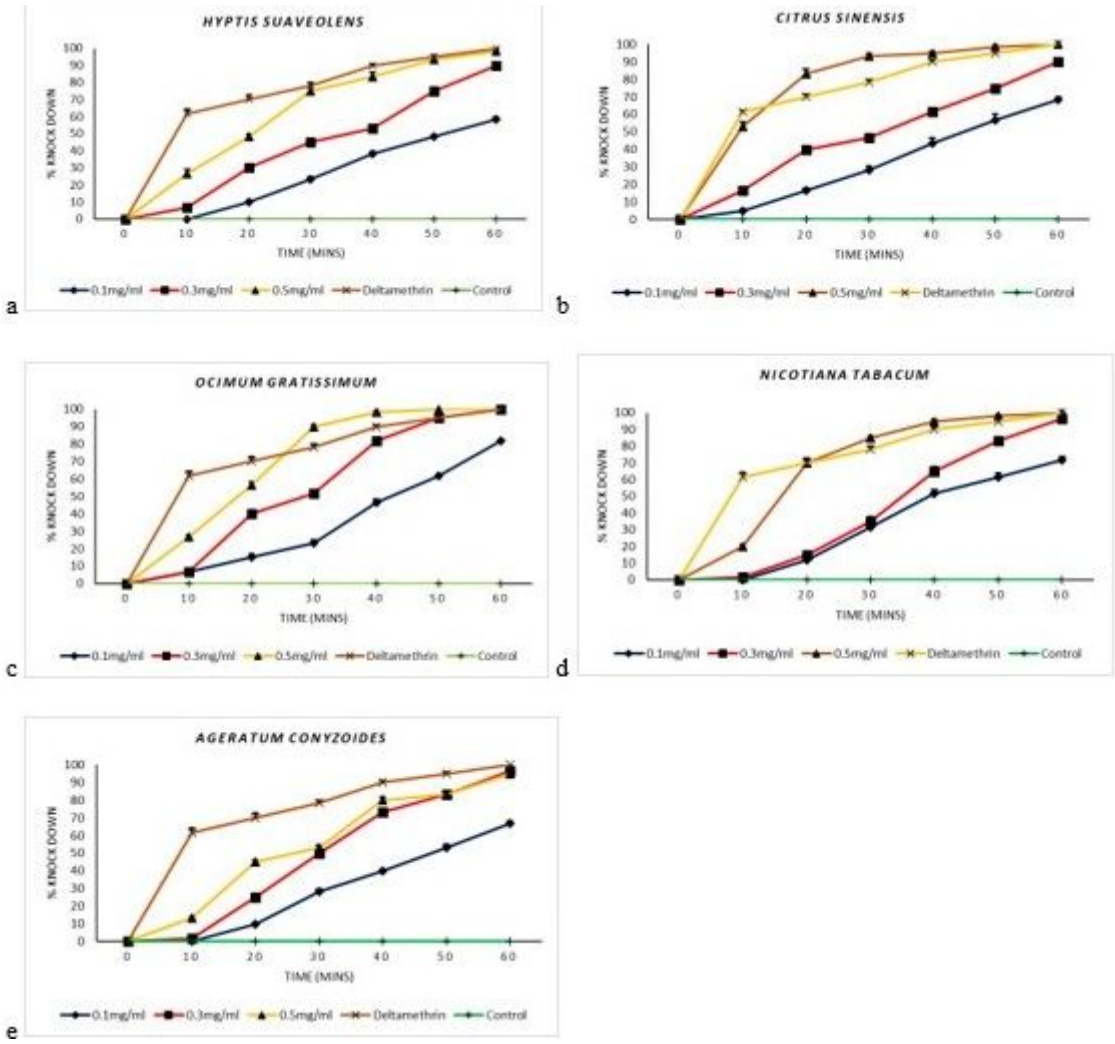

Figure 1

Percentage knockdown of *Anopheles gambiae* exposed to oils from (a) *Hyptis suaveolens* (b) *Citrus sinensis* (c) *Ocimum gratissimum* (d) *Nicotiana tabacum* and (e) *Ageratum conyzoides*